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# DNA Strand Break Repair and Neurodegeneration

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## Abstract

A number of DNA repair disorders are known to cause neurological problems. These disorders can be broadly characterised into early developmental, mid-to-late developmental or progressive. The exact developmental processes that are affected can influence disease pathology, with symptoms ranging from early embryonic lethality to late-onset ataxia. The category these diseases belong to depends on the frequency of lesions arising in the brain, the role of the defective repair pathway, and the nature of the mutation within the patient. Using observations from patients and transgenic mice, we discuss the importance of double strand break repair during neuroprogenitor proliferation and brain development and the repair of single stranded lesions in neuronal function and maintenance.

## 1 Introduction

The genome is constantly under attack from endogenous and exogenous genotoxic agents, and also possesses an inherent level of instability [1]. Breaks can arise in one or both DNA strands, and chemical adducts or crosslinks can arise on or between DNA bases [2-4]. In addition, the breakage and repair of cellular DNA is also a necessary part of several cellular processes critical for cellular growth and proliferation and for organismal development. Genome stability and maintenance requires a number of overlapping biochemical pathways involving many different proteins, clustered into specific DNA repair pathways. Loss of function of these proteins can lead to a variety of disorders, with pathologies including growth and developmental defects, immunodeficiency, cancer, neurodegeneration and ageing [5-7]. The association of DNA repair defects with both elevated predisposition to cancer and to increased rates of neurodegeneration and ageing, sometimes in the same genetic disease, is particularly intriguing, because cancer is a disease of excessive cell growth and survival, whereas neurodegeneration is a disease of excessive cell dysfunction and death. Opposite cellular end points can thus arise from defects in common or related processes [8]. In this review we focus on DNA damage defective diseases associated with neurological dysfunction, and attempt to rationalise the differences in underlying molecular defects between developmental and neurodegenerative pathologies.

## 2 Brain development

Embryonic development involves waves of rapid cell division throughout the developing embryo, followed by complex periods of migration and differentiation (Figure 1) [9]. During this time there is a requirement for rapid and efficient mechanisms to fix transcription- and replication-associated DNA breaks as well as naturally occurring oxidative breaks. Failure to repair these breaks may lead to the accumulation of damage and cell death, resulting in neuronal loss at different stages of development, depending on the frequency of DNA lesions and DNA damage signalling pathways.

Cortical development begins from around 5-7 gestation weeks in humans, during which there is a rapid proliferation of neuroprogenitor cells from the ganglionic eminence (GE) of the ventricular zone (VZ) through the neocortex [9, 10]. During this phase, there are tightly coordinated processes of cell division, differentiation and migration from the site of neurogenesis to the cortical plate,

resulting in the generation of neurons that populate the neocortex [11, 12]. The brain then undergoes a second phase of rapid neurogenesis during the latter phase of development. In humans this occurs from 12 weeks gestation continuing through until around 6 months after birth [13, 14]. During this phase there is limited cell division within the VZ, but a burst of cell division, migration and differentiation is initiated in the subventricular zone (SVZ) to generate a subpopulation of late-born cortical neurons and interneurons [10]. In primates including humans this stage of development is greatly expanded to generate the huge numbers of neuronal cells that populate the cortex, suggesting that humans may be more susceptible to mutations in DNA repair defects than rodent models [15]. Early cerebellar development follows a similar pattern of proliferation from the VZ at around 9-10 weeks to form the Purkinje cell layer, but late-born granule cells are formed from 16 weeks, originating in the external granule layer (EGL) and moving inwards to the granule layer past the maturing Purkinje cells. This wave of neurogenesis continues until around 7 months after birth [9].

### 3 Double strand break repair and neurogenesis

Double strand break repair (DSBR) involves two distinct pathways (Figure 2) [16]: During G1 and early S-phase, the dominant repair pathway involves the processing and ligation of non-homologous DNA ends (non-homologous end joining; NHEJ). The principal components of NHEJ are DNA-dependent protein kinase (DNA-PK), XRCC4, DNA ligase 4 (Lig4) and XLF/Cernunnos. Damaged DNA termini are processed primarily by PNKP, TDP2, Artemis, aprataxin, or by one of several nucleases, which prepare the DNA ends for ligation by Lig4 [17, 18]. Because of the processing events that occur during NHEJ, loss of genetic information can occur. However, chromosomes are duplicated during S-phase, and double strand breaks (DSBs) occurring in replicated DNA during S/G2 can be repaired without loss of information by recombination between the damaged sister chromatid and its homologous undamaged counterpart. This process, denoted homologous recombination (HR), requires the activity of a number of proteins including BRCA1, BRCA2, XRCC2, XRCC3, and RAD51. HR is initiated by nucleolytic resection of 5'-termini at DSBs in a CtIP/MRN-dependent manner, after which RAD51 facilitates invasion of the single-stranded 3'-tail into the homologous sister chromatid, thereby enabling accurate repair of the DSB [19].

#### 3.1.1 Non homologous end joining, Lig4 and NHEJ1 syndromes

Cells of the developing brain may switch in dependence from one DNA repair pathway to another, depending on the phase of the cell cycle they are in (Figure 2) [20, 21]. In the early proliferative phase there is a greater requirement for repair by HR, with the advantage that genetic information is conserved. Loss of HR leads to embryonic lethality at a very early stage [22]. It is rare that homozygous HR mutants survive to birth, and are usually only seen in patients with hypomorphic mutations that retain sufficient HR activity to progress through this developmental stage. Patients with such mutations suffer a spectrum of developmental disorders (which can include microcephaly) such as those seen in Fanconi anaemia [23].

During migration and differentiation there is a greater dependence the NHEJ pathway, since cells exiting the cell cycle and undergoing neuronal differentiation are in G1/G0. With no sister chromatid available to undergo HR, NHEJ is the only DSB repair pathway available. Mutations in the NHEJ pathway, therefore result in the loss of neuroprogenitor cells, loss of cortical neurons, and microcephaly. The diseases associated with microcephaly listed in Figure 2 (green boxes) illustrate

that the latter phase of human brain development is particularly sensitive to loss of NHEJ capacity. The structural components of the core NHEJ complex, KU70 and KU80, are essential for human cell viability [24, 25] and there are no documented patients with mutations in these proteins. Other components of the NHEJ complex (DNA-PKcs, Lig4 and XLF/Cernunnos) are also likely to be essential for development, although patients carrying hypomorphic mutations in these proteins have been reported [26-28]. Patients with Lig4 syndrome or *severe combined immunodeficiency with microcephaly, growth retardation, and sensitivity to ionizing radiation* (NHEJ1 syndrome; mutated in XLF/Cernunnos) suffer from microcephaly at birth and immunodeficiency, consistent with an essential role for NHEJ in both brain development and VDJ recombination.

Mice with attenuated NHEJ show some symptoms consistent with those seen in humans, including growth defects and immunodeficiency [29, 30]. Complete loss of NHEJ ligation factors XRCC4 and LIG4 results in embryonic lethality in the latter stages of development, showing loss of cells following defective lymphogenesis and massive apoptotic loss of neuronal cells in the cortex and hindbrain [31-33]. The cause of neuronal loss is due to DSBs arising during the VZ and SVZ proliferative phases and persisting during differentiation within the intermediate zone (IZ) [22, 33].

### 3.1.2 ATM and Ataxia telangiectasia

The PI 3-kinase-related kinases ATM and ATR detect genomic DSBs and phosphorylate a range of targets (reviewed in [34, 35]) including cell cycle checkpoint proteins CHK1 and CHK2. These targets suppress DNA replication origin firing and cell cycle progression, allowing cells to repair DSBs before proceeding with cell division [36, 37]. Ultimately, if a number of DSBs persist then these kinases will signal apoptosis by phosphorylation and stabilisation of p53. This is likely why neuronal loss in mouse NHEJ mutants can be rescued by inactivation of ATM or p53 [38-40]. Since ATM signals cell death in neurons with unrepaired DSBs, it is somewhat paradoxical that whilst loss of ATM activity rescues the embryonic lethality of NHEJ-deficient mouse mutants, it results in ataxia telangiectasia (A-T) in an otherwise normal background in humans.

A-T patients display symptoms indicative of compromised responses to DSBs, such as immunodeficiency, sterility, radiosensitivity and cancer predisposition; the severity of the disease usually correlating with the nature of the mutation, or the amount of active ATM protein within the cells of the patient [41, 42]. Progressive neurodegeneration and ataxia is arguably the pathology that impacts on quality of life most severely, though patients typically die from respiratory infections or cancer [43]. Whereas NHEJ disorders appear to result from neuronal loss during corticogenesis, loss of ATM function impacts primarily on neuronal maintenance in the cerebellum, brain stem and spinal cord, with cell loss being particularly severe in the Purkinje cells, granule cells and molecular layer [44-46]. The degenerative pathology of A-T may reflect the loss of ATM-mediated DSB signalling during neurodevelopment and maintenance, and thus persistence of dysfunctional neural cells into the adult brain that would otherwise commit to apoptosis, and/or may reflect the requirement for ATM for repair of a subset of DSBs in neurons. In any case, loss of ATM results primarily in neuronal dysfunction and ataxia, rather than developmental microcephaly. However, it has been difficult to establish the precise consequence of ATM loss in the cerebellum, since *Atm*<sup>-/-</sup> mice display only very mild, if any neurological defects [47, 48]. This suggests that the human brain has a higher dependence on ATM than that of rodents.

### 3.1.3 ATR and ATR-Seckel syndrome

While ATM is activated by chromosomal DSBs, ATR is activated by stretches of RPA bound to single-stranded DNA surrounding collapsed replication forks [49] and plays an important role in the recovery from replication stress [50, 51]. The neurological phenotype of patients with ATR mutations overlaps with NHEJ mutants more closely than does that of patients with mutations in ATM. This may reflect a higher level of DNA damage-induced cell death in proliferating neurons during neuronal development. Seckel syndrome shows characteristic growth defects, microcephaly and mental retardation. However, the lack of severe radiosensitivity and immunological defects distinguishes the mechanism of this disease from those described above [52, 53]. In mice, ATR is essential for development [54, 55], but those carrying a Seckel syndrome mutation in humanized ATR (ATR<sup>S</sup>) show the characteristic “bird-head” microcephaly seen in human Seckel syndrome patients. In this model, the severe loss of neurons throughout the cerebellum, hippocampus and cortex correlates with areas of neurogenesis in the developing brain, with cell death arising from replication stress in the absence of ATR activity [56]. Similarly, a brain-specific ATR knockout suggested that ATR signalling in the brain is critical during rapid waves of neuroprogenitor cell division in the GE and cerebellum at late stages of development [57]. Other mutant proteins, such as pericentrin and MCPH1 have been implicated in the cause of microcephaly through coupling ATR signalling to the G2/M checkpoint via the centrosome [58, 59]. More specifically it is thought that ATR/Chk1 signalling maintains neuronal stem cell populations during these developmental phases by regulating the orientation of the mitotic spindle and controlling symmetric vs. asymmetric cell division in the neuroprogenitor population [60] although other models for the role of centrosome in brain development have been suggested [61].

### 3.1.4 The MRN complex, Nijmegen breakage syndrome and Ataxia telangiectasia-like disorder

An important mediator of the DSB response is the MRN complex, comprising the proteins MRE11, NBS1 (NBN) and RAD50. This complex is required for tethering the two ends of the DSB, nucleolytic resection, and mediating the ATM/ATR damage response [62-66]. A number of hypomorphic mutations in NBS1, MRE11 and RAD50 have been identified in patients with Nijmegen breakage syndrome (NBS), A-T-like disorder (A-TLD) and NBS-like disorder (NBSLD) respectively. Patients with these mutations show a phenotypic spectrum similar to other NHEJ disorders (microcephaly, immunodeficiency, radiosensitivity and cancer predisposition) with varying severity that may reflect the nature of the hypomorphic mutation on protein levels and DSB repair activity, or functional differences between these proteins [67-72].

Because of MRN's involvement in HR, NHEJ and damage signalling, it is not surprising that complete loss of these components in mice results in early embryonic lethality [73-75]. Pathologies that replicate some aspects of NBS and A-TLD have been seen in hypomorphic Nbs1 and Mre11 mouse mutants, but these do not show neuropathology [76-78]. Shull et al attempted to address why NBS results in microcephaly, whereas A-TLD results in an “AT-like” neurodegenerative phenotype [78]. Using ionising radiation (IR) or brain-specific Lig4 deletion (Lig4<sup>Nes-Cre</sup>) as a source of DSB damage, a higher level of ATM and p53-dependent apoptosis was observed in mice mimicking NBS than those mimicking A-TLD. This may indicate a more important role for Mre11 than Nbs1 in apoptotic signalling via ATM, and thus greater impact on cell death during neurodevelopment, and/or that or that loss of MRN activity is greater in mice mimicking A-TLD than those mimicking NBS [78].

In summary, the disorders described above illustrate the possible consequences of unrepaired DSBs in the brain (figure 3). In cycling neuroprogenitors, ATR activates cell cycle checkpoints during replication stress and mitosis and so reduced activity of this protein kinase may lead to excessive DNA damage and cell death during neurodevelopment, resulting in microcephaly. Similarly, in NHEJ-defective diseases, the elevated level of unrepaired DSBs may similarly lead to cell death and microcephaly during neurodevelopment, particularly during G0/G1 in differentiating neurons, in which HR-mediated repair is unavailable. In such cells, cell death occurs via ATM signalling, mediated by the MRN complex. Consequently, if ATM signalling fails, neurons with unrepaired DSBs may escape apoptosis and perhaps persist in a dysfunctional state, contributing to juvenile neuropathology. The causes of late-onset progressive neuropathology in patients with A-T and A-TLD are still under debate, but are probably due to the cumulative impact of defective DNA damage signalling and repair during the lifetime of post-mitotic neurons [79].

## **4 Progressive neurodegeneration and cerebellar ataxia**

Whilst DSBs are severe lesions that impact greatly on proliferating and differentiating cells, and consequently on neurodevelopment, these lesions are relatively rare. In contrast, lesions on a single strand of DNA, and in particular single-strand breaks (SSBs), arise 3 orders of magnitude more frequently. Single-strand lesions are normally repaired rapidly by the SSBR and TC-NER pathways (Figure 4), but if these pathways are defective, long-lived single-strand lesions can trigger cell death by blocking the progression of RNA polymerases during transcription [80, 81]. This can impact on proliferating cells, but the lesions can also be readily and accurately dealt with by specific repair processes in S/G2 [82], and/or the damaged cells can be removed and readily replaced in regenerative tissues. Consequently, defects in the repair of single-strand lesions are less likely to cause developmental defects or microcephaly, unless the repair defect is so severe that alternative repair and regenerative processes are overwhelmed. In post-mitotic cells, alternative S/G2 repair pathways are absent, and the pathways shown in figure 4 are the primary if not only repair pathways available. Consequently, attenuation or loss of those repair processes may result in degeneration of non-cycling tissues. This perhaps explains the link between defects in the repair of single-strand lesions, neurodegeneration, and premature aging.

### **4.1 NER, Cockayne syndrome and Xeroderma Pigmentosum**

Proteins defective in the CS and XP complementation groups are collectively responsible for the removal of cyclobutane pyrimidine dimers (CPDs), 6-4 photoproducts (6-4 PPs), bulky adducts and oxidative base damage from the DNA using nucleotide excision repair (NER) [83-85]. In non-transcribed regions, these helix-distorting lesions are detected by HR23B-XPC and DDB2 and repaired by Global Genome Repair (GG-NER) [86]. Patients with defects in this pathway show the characteristic UV sensitivity and skin cancer associated with Xeroderma Pigmentosum (XP), but these patients rarely show neurological symptoms [87]. Conversely, if these lesions lie in transcribed regions of the genome, RNA Polymerase stalling signals the recruitment of repair factors in the transcription-coupled NER (TC-NER) pathway. Components specific to processing stalled RNA polymerase and TC-NER are CSA and CSB, with subsequent steps common to both NER pathways and involving unwinding DNA around the lesion (by XPB/XPD), excising a region of DNA flanking the lesion (by ERCC1-XPF/XPG), and DNA synthesis using the complementary (non-damaged) strand as a template (by PCNA/Pol  $\delta/\epsilon/\kappa$ ) [88-92]. Patients with defective TC-NER do not develop cancer, but



suffer from XP, Cockayne's syndrome (CS), or trichothiodystrophy (TTD). These result in a spectrum of neurological phenotypes, including microcephaly, progressive neurological impairment, ataxia, brittle hair and ichthiosis with variable onset and severity [93-97].

Although CS and XP mouse models initially failed to reproduce the neurodegenerative symptoms seen in patients [98-101], combining NER mutants leads to a more severe neurological phenotype that specifically targets the cerebellum during post-natal development [102, 103]. For example, *Xpa<sup>-/-</sup>/Csb<sup>-/-</sup>* mice develop ataxia within 1 week of birth following decreased cell proliferation and increased apoptosis in the external granular layer of the cerebellum [103].

## 4.2 Single-strand break repair (SSBR)

Single-strand break repair (SSBR) has evolved in vertebrates to detect and repair the many thousands of nicks and single-stranded gaps caused by cellular genotoxic events [104]. Deficiency in SSBR leads to cellular sensitivity to radiation, oxidative stress and base damaging agents [105, 106]. PARP1 is the sensor of chromosomal SSBs, and results in accumulation of SSBR proteins such as the XRCC1/DNA Ligase III (Lig3) heterodimer at sites of DNA damage [107, 108]. XRCC1/Lig3 appears to assemble or recruit a number of DNA processing factors at sites of SSBs, such as Aprataxin, TDP1, PNKP, and DNA pol  $\beta$ , [109-111]. In mice, Lig3 is essential for mitochondrial DNA maintenance and embryonic viability [112, 113]. Similarly, XRCC1 is required for embryonic viability [114], though most likely for its role in nuclear DNA repair [115, 116]. Brain-specific knockout of XRCC1 (*Xrcc1<sup>Nes-Cre</sup>*) results in the severe loss of neurons in the cerebellum and hippocampus. Progressive loss of proliferating and differentiating interneurons in the EGL of the cerebellum demonstrates a requirement for SSBR in postnatal brain development and maintenance. Progression of this disease is rapid, with mice developing ataxia from several weeks old and dying at around 4 months, most likely as a result of episodic seizures [117]. As discussed above, this likely reflects the importance of *Xrcc1* for repair of all types of SSB, and thus the saturation of HR capacity during S phase in *Xrcc1*-defective proliferating cells, during neurodevelopment.

### 4.2.1 Ataxia with oculomotor apraxia-1 (AOA1)

Patients with ataxia resulting from mutant XRCC1 have yet to be found, but there are several cases of cerebellar ataxia resulting from less severe defects in SSBR. Neurological symptoms resemble those seen in *Xrcc1<sup>Nes-Cre</sup>* mice, and overlap with those of A-T patients. These diseases can be distinguished from A-T, however, because patients do not display extra-neurological features. The most common disorder associated with defects in SSBR is ataxia oculomotor apraxia-1 (EAOH or AOA1), which exhibits progressive cerebellar ataxia from 3-10 years. The disease is caused by the loss of functional aprataxin [118, 119], a protein that interacts directly with XRCC1 via an amino-terminal FHA domain. APTX is involved in the end processing step of SSBR (see Fig.4), during which it removes AMP from the 5'-termini of DNA breaks resulting from abortive DNA ligation events [120-122]. Aprataxin-deficient neuronal cells are deficient in short-patch SSBR [122] and show sensitivity to genotoxic agents [123]. It should be noted that APTX also interacts with XRCC4, via its FHA domain, and thus is most likely also involved in DSB repair by NHEJ. However, to date, a defect in the repair of cellular DSBs in APTX defective cells has not been observed [122, 124]. Unfortunately, similar to A-T, the neurodegenerative pathology observed in AOA1 patients is not recapitulated in *Aptx<sup>-/-</sup>* mice, highlighting the difficulty in studying progressive DNA damage-induced neurodegeneration in the mouse.



#### 4.2.2 Spinocerebellar ataxia with axonal neuropathy (SCAN1)

Spinocerebellar ataxia with axonal neuropathy (SCAN1), results in cerebellar ataxia from around 15 years old, and caused by the loss of functional tyrosyl-DNA phosphodiesterase 1 (TDP1). This protein interacts with XRCC1/Lig3 heterodimer via direct interaction with Lig3, and is also involved in the end processing step of SSBs [125]. TDP1 removes trapped topoisomerase peptides from 3'-termini of DNA breaks resulting from abortive topoisomerase I (TOP1) activity [125, 126], and is also active on other types of 3'-modified termini [127, 128]. Loss of this protein results in reduced rates of cellular SSB repair and sensitivity to a range of genotoxic agents, but to date has not been reported to result in reduced rates of DSB repair [125, 129]. Tdp1 <sup>-/-</sup> mice show late-onset progressive atrophy in the cerebellum, consistent with a SCAN1 phenotype [130].

Mechanistically similar to TDP1, tyrosyl-DNA phosphodiesterase 2 (TDP2) acts at sites of aborted Topoisomerase II (TOP2) and processes phosphotyrosyl-peptides on the 5'-ends of DSBs [131]. There are so far no clinical phenotypes for defective processing of these lesions, and Tdp2 <sup>-/-</sup> do not show severe neurological symptoms, but a requirement for processing stalled TOP2 during replication and/or transcription could result in SCAN1-like symptoms in older TDP2 patients.

#### 4.2.3 Microcephaly with Early-Onset Seizures and Developmental Delay (MCSZ)

Polynucleotide kinase/phosphatase (PNKP) resembles aprataxin in the presence of an N-terminal fork-head associated (FHA) domain that is required for direct interaction with XRCC1 and XRCC4 [110, 132]. PNKP is another DNA end processing factor that processes damaged termini at DNA strand breaks. In this case the DNA substrates are 5'-hydroxyl and/or 3'-phosphate termini, at either SSBs [110, 133] or DSBs [132, 134], which require the DNA kinase or DNA phosphatase activity of PNKP to restore ligatable 5'-phosphate and 3'-hydroxyl termini, respectively [135]. In a similar manner to aprataxin and TDP1, loss of PNKP phosphatase activity results in delayed repair of cellular SSBs, and sensitivity to oxidative stress [109, 136]. Patients with mutations in this protein suffer from microcephaly, early-onset seizures and developmental delay (MCSZ [137]). The spectrum of neurological symptoms in these patients could represent a very severe SSB repair defect that is reminiscent of *Xrcc1<sup>Nes-Cre</sup>* mice with an onset period in late development. This would be consistent with the observation that ~70% of SSBs arising from endogenous oxidative stress are likely to be substrates for PNKP [135]. However, it could also reflect the overlapping requirement for PNKP activity during NHEJ, and thus during neuroprogenitor proliferation. A similar argument could be made for Tdp1 and APTX, since both of these arguably also can function at sub-sets of DSBs [121, 132]. However, as discussed above, there is little evidence for a defect in DSB re-joining in cells lacking either APTX or TDP1 [122, 124, 125]. To date, therefore, neurodegeneration in SCAN1 and AOA1 is most readily explained by the defect in SSB repair, whereas the neurodevelopmental/seizure pathology observed in MCSZ might reflect a more severe defect in SSB repair and/or combined defects in both SSB repair and DSB repair.

### 4.3 Single-strand lesions and neuronal cell death

Single-strand lesions are not believed to activate apoptotic pathways directly, but accumulation of CPDs, 6-4 PPs, stalled topoisomerase, SSBs or abasic sites in transcribed DNA regions can slow or block RNA polymerases [138-142]. There are significant phenotypic overlaps between the SSB repair diseases such as AOA1 and SCAN1 and those resulting from defective RNA processing such as ataxia-oculomotor apraxia-2 (AOA2) [143, 144], supporting the idea that certain neuronal cells are sensitive to transcription stalling. Furthermore it has recently been reported that RNA processing and DNA

repair are closely linked at stalled RNA polymerase sites, suggesting a mechanistic link between these two processes as well as a clinical one [145-147]. The timing and progression of the neurological pathologies described above varies from case to case, and likely involves numerous factors including the endogenous level of the specific lesion(s) processed by the relevant protein, the severity of the mutation, and the level of transcriptional activity within the affected cells or tissues. High levels of transcription may increase the susceptibility of particular cells in the brain to apoptosis via stalled transcription, and may explain why particular sensitivity in the cerebellum leads to ataxia.

Accumulation of transcription-blocking lesions could result in the depletion of essential mRNAs and eventually lead to cell death by a number of indirect means, but alternatively cell death may be triggered directly via activation of specific sensors and effectors from these lesions (Figure 3). For example, stalled RNA polymerase II may stimulate p53-mediated apoptosis via ATR, even in the absence of replication [148-150], possibly via accumulation of RPA on regions of single-stranded DNA [151]. Activation of PARP at SSBs can trigger another mechanism of cell death. Prolonged activation leads to accumulation of cellular poly(ADP-ribose) (PAR) and depletion of NAD<sup>+</sup>, which can stimulate the release of apoptosis-inducing factor from mitochondria, resulting in a PARP-dependent, p53-independent, form of cell death known as *parthanatos* [152-154]. Neuronal cells seem to be particularly sensitive to PARP-induced cell death, as illustrated in models of cerebral ischemia [155, 156]. Again, high levels of oxidative stress and PARP activity may confer hypersensitivity of certain regions of the brain to single-strand damage.

## 5 Conclusion

Recent data from transgenic models has enabled us to resolve the overlapping neurological phenotypes in DNA repair disorders. As illustrated in figure 1, these disorders can be categorised into early developmental (embryonic lethality) mid-to-late developmental (microcephaly) or post-developmental (neurodegenerative). Broadly-speaking, these categories are defined by the repair pathway that is defective, with a requirement for HR particularly evident during early development, NHEJ during late development, and repair of single-strand lesions for post-developmental maintenance. However, exceptions to this general rule are evident. For example, very severe defects in SSBR such as those observed in *Xrcc1*-defective mice, and possibly in PNKP-defective MCSZ patients, may saturate alternative (homologous recombination-mediated) repair mechanisms in proliferating cells and thereby result in neurodevelopmental defects and microcephaly. In addition, ATM and MRE11 are involved in DSBR, yet the neural phenotypes of A-T and A-TLD patients are reminiscent of individuals with defects in the repair of single-strand lesions. Whilst at first glance this may appear perplexing, there are two possible explanations for this: 1: There may be an as yet undefined role for ATM and MRN in the repair of single-strand lesions at blocked transcription sites, or 2: Deficient ATM signalling enables damaged neurons to escape apoptosis with persistent transcription-blocking lesions, and accumulation of these eventually leads to cell death by the alternative mechanisms described above. Either of these could contribute to the progressive degenerative pathology similar to that of diseases in which the repair of single-strand lesions is defective.

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## 7 Figure legends

**Figure 1: A model for DNA repair during corticogenesis.** A schematic representation of human fetal cerebral wall sections taken during cortical development at the times shown. Early embryogenesis depends heavily on homologous recombination (HR) within dividing cells (blue) during rapid growth phases. Waves of neuroprogenitor division begin from 5-7 weeks within the ventricular zone (VZ) weeks, and from 12 weeks-6 months after birth in the subventricular zone (SVZ). The red curve corresponds to waves of migration and differentiation through the intermediate zone (IZ) to the cortical plate (CP). G1/G0 neuronal cells (red) are dependent on NHEJ for DSBR. Following differentiation, DSBs are rare and neuronal cells become increasingly dependent on SSBR/TC-NER to repair frequently occurring single-strand lesions. (Figure adapted from Sidman and Rakic, 1973).

**Figure 2: Double strand break repair.** Figure shows simplified pathways illustrating the core components of non-homologous end joining (NHEJ) and homologous recombination (HR). Pathway choice depends primarily on cell cycle, since HR requires homologous DNA in the sister chromatid to undergo recombination and can only occur in late S/G2, but NHEJ can occur in G1/early S. Green boxes show microcephalies resulting from defects in the indicated proteins: Nijmegen Breakage Syndrome (NBS), NBS-like disorder (NBS-LD), microcephaly, early-onset seizures and developmental delay (MCSZ), NHEJ1 syndrome, Lig4 Syndrome, Microcephaly 1 (MCPH1), ATR-Seckel Syndrome and Fanconi anemia complementation group D1 (FANCD1). Yellow boxes show ataxias: Ataxia telangiectasia (A-T) and A-T-like disorder (A-TLD).

**Figure 3: Mechanisms of cell death from unrepaired breaks in neuronal cells.** Persistent lesions can exist as a double strand break (DSB), intrastrand crosslinks (CPD or 6-4 PP), damaged base or single strand break (SSB). DSBs activate ATM and p53-mediated apoptosis, or via ATR if the cell undergoes replication (during neurogenesis, for example). Transcription-blocking lesions stall RNA polymerase, which activates ATR and induces apoptosis via p53, plus there may be indirect effects from depletion of essential RNAs. In the case of SSBs “*parthanatos*” is an alternative, p53-independent mechanism of cell death, in which PARP generates poly(ADP) ribose (PAR), which stimulates mitochondria to release apoptosis inducing factor (AIF).

**Figure 4: Repair of single stranded lesions.** Figure shows a simplified model for single strand break repair (SSBR) and transcription-coupled nucleotide excision repair (TC-NER). The green box shows a microcephaly (MCSZ) and yellow boxes show ataxias resulting from defects in the indicated proteins: Cockayne Syndrome, type B (CSB), ataxia with oculomotor apraxia 1 (AOA1), spinocerebellar ataxia with axonal neuropathy (SCAN1) and Trichothiodystrophy (TTD).

Figure 1:

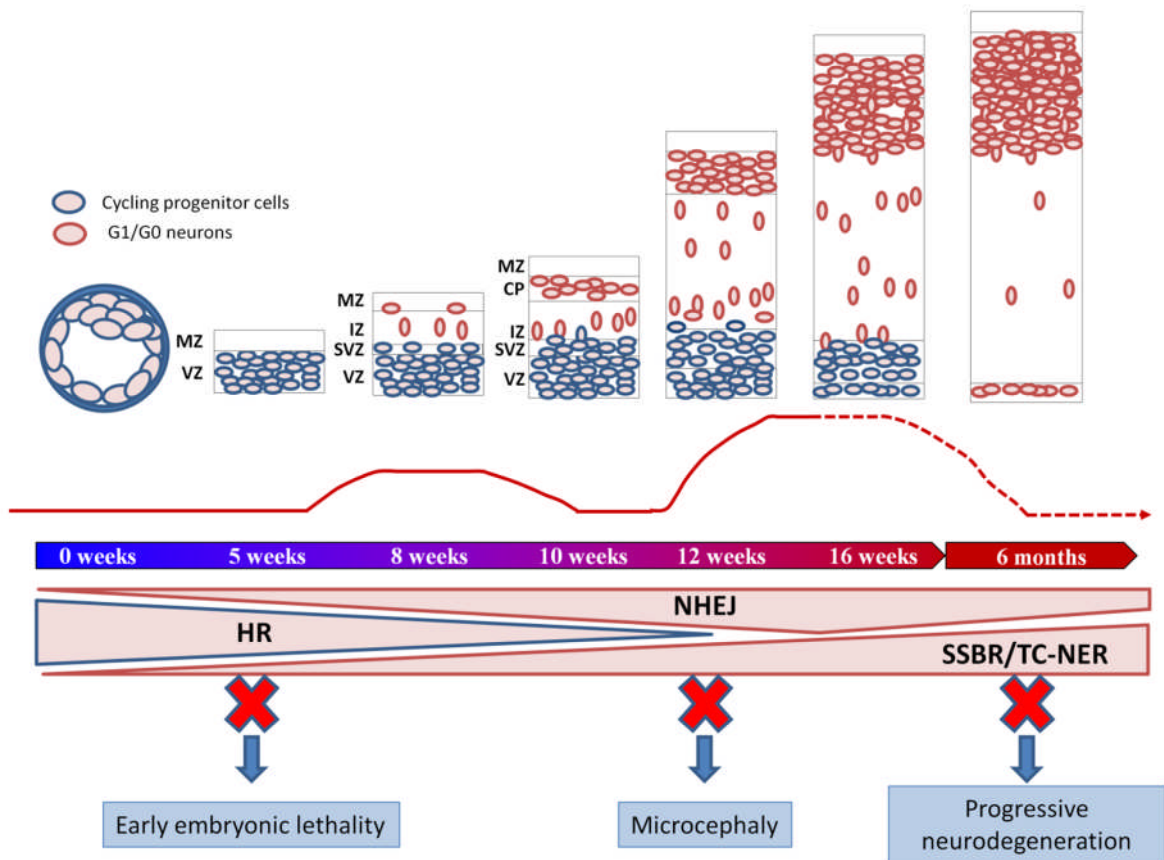
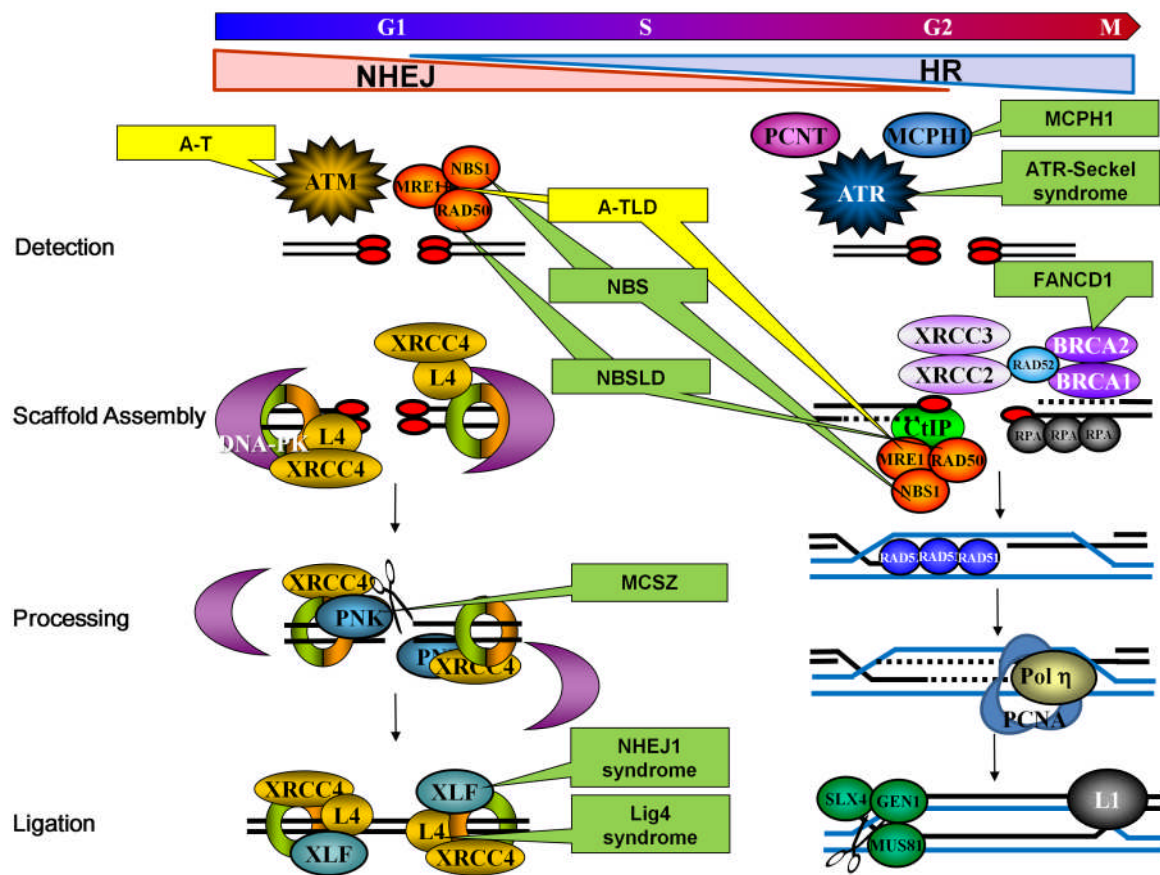


Figure 2





**Figure 3**

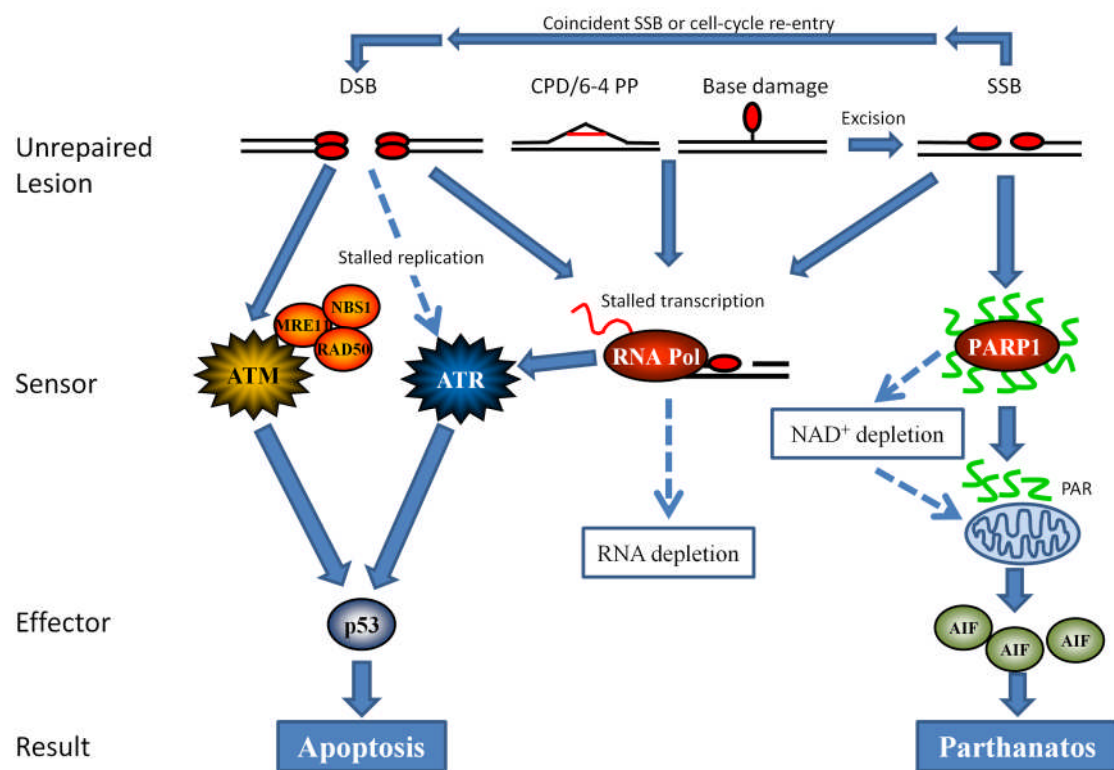


Figure 4

